

This listing of claims will replace all prior versions, listings, of claims in the application:

**Listing of Claims:**

1. (original) A bio-fuel cell system, comprising:
  - a) a fuel cell including a cathode compartment containing a cathode electrode with an aqueous solution containing ferric ions ( $Fe^{3+}$ ) being circulated into said cathode compartment with a reaction at the cathode electrode being reduction of ferric ions at the cathode electrode in a reaction given by  $4Fe^{3+} + 4e^- = 4Fe^{2+}$ ;  
an anode compartment containing an anode electrode with a fuel having a hydrogen constituent being pumped into said anode compartment, said anode compartment being separated from said cathode compartment by a membrane permeable to protons, a reaction at the anode electrode being electrochemical oxidation of the fuel to produce electrons ( $e^-$ ) and protons ( $H^+$ ), wherein protons ( $H^+$ ) formed by the oxidation of hydrogen cross the proton exchange membrane into the cathode compartment; and
  - b) a bioreactor containing chemolithotrophic microorganisms, a pump for pumping a fluid containing oxygen ( $O_2$ ) and carbon dioxide into the bioreactor, the bioreactor being in flow communication with the cathode compartment so that the aqueous solution containing ferrous ions ( $Fe^{2+}$ ) and protons ( $H^+$ ) is circulated from the cathode compartment to the bioreactor where the ferrous ions ( $Fe^{2+}$ ) are oxidized by the chemolithotrophic microorganisms to ferric ions ( $Fe^{3+}$ ) in an aerobic oxidation reaction given by  $4Fe^{2+} + 4H^+ + O_2 = 4Fe^{3+} + 2H_2O$ , wherein electrical power is obtained by making electrical connection between a load and the anode and cathode electrodes, and including a pump for pumping a fluid containing ferric ions ( $Fe^{3+}$ ) into said cathode compartment.
2. (original) The bio-fuel cell system according to claim 1 wherein the membrane permeable to protons is a proton exchange membrane.

3. (original) The bio-fuel cell system according to claim 1 wherein the membrane permeable to protons is made of a substantially inert material having pores extending therethrough less than about 10 micrometers in diameter.
4. (currently amended) The bio-fuel cell system according to claim 1, ~~2 or 3~~ wherein the bioreactor and the cathode compartment contain dissolved nutrients for facilitating growth of the chemolithotrophic microorganisms.
5. (original) The bio-fuel cell system according to claim 4 wherein the dissolved nutrients is one or more of ammonium sulfate, potassium phosphate, magnesium sulfate, potassium chloride, calcium nitrate, calcium chloride and sulfuric acid.
6. (currently amended) The bio-fuel cell system according to claim 1, ~~2, 3, 4 or 5~~ wherein the fuel having a hydrogen constituent is selected from the group consisting of hydrogen gas, methanol, methane and ethanol.
7. (currently amended) The bio-fuel cell system according to claim 1, ~~2, 3, 4 or 5~~ wherein the fuel having a hydrogen constituent is hydrogen gas ( $H_2$ ), and wherein the electrochemical oxidation reaction is oxidation of hydrogen at the anode electrode in a reaction given by  $2H_2 = 4H^+ + 4e^-$ , and so that an overall bio-fuel cell reaction is given by  $2H_2 + O_2 = 2H_2O$ .
8. (currently amended) The bio-fuel cell system according to claim 1, ~~2, 3, 4, 5, 6 or 7~~ wherein the chemolithotrophic microorganisms are *Acidithiobacillus ferrooxidans*.
9. (currently amended) The bio-fuel cell system according to claim 1, ~~2, 3, 4, 5, 6 or 7~~ wherein the chemolithotrophic microorganisms are selected from the group consisting of *Leptospirillum ferrooxidans*, *Acidimicrobium*, *Alicyclobacillus*, and *Sulfobacillus*.

10. (currently amended) The bio-fuel cell system according to claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 wherein the cathode electrode is made from a chemically inert electrically conducting material.

11. (original) The bio-fuel cell system according to claim 10 wherein the cathode electrode includes a layer of a porous material selected from the group consisting of carbon, nickel and stainless steel.

12. (original) The bio-fuel cell system according to claim 10 wherein the cathode electrode includes a solid plate of a material selected from the group consisting of carbon, nickel and stainless steel.

13. (currently amended) The bio-fuel cell system according to claim 10, 11 or 12 wherein the cathode electrode includes a catalyst.

14. (original) The bio-fuel cell system according to claim 13 wherein the catalyst is one of gold, platinum, palladium and lead.

15. (currently amended) The bio-fuel cell system according to claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 wherein the bioreactor is a vessel in flow communication with the cathode compartment and enclosing the chemolithotrophic microorganisms, and wherein the aqueous solution containing ferric ions ( $Fe^{3+}$ ) is circulated into said cathode compartment, including a pump for circulating the aqueous solution containing ferrous ions ( $Fe^{2+}$ ) and protons ( $H^+$ ) produced in the cathode compartment between the cathode compartment and the bioreactor, where the ferrous ions ( $Fe^{2+}$ ) are oxidized by the chemolithotrophic microorganisms to ferric ions ( $Fe^{3+}$ ) in said aerobic oxidation reaction, and wherein the ferric ions are recirculated back to the cathode compartment.

16. (currently amended) The bio-fuel cell system according to any one of claims 1 to 15 claim1 wherein the fluid containing oxygen ( $O_2$ ) pumped into the bioreactor includes carbon dioxide ( $CO_2$ ) for production of biomass.

17. (original) The bio-fuel cell system according to claim 16 including voltage control means for applying and controlling a voltage on the cathode electrode for controlling a ratio of electrical production to biomass production by varying microbial cultivation parameters.
18. (original) The bio-fuel cell system according to claim 16 including reagent control means for controlling a ratio of  $\text{Fe}^{2+}/\text{Fe}^{3+}$  concentrations for varying microbial cultivation parameters in order to control a ratio of electrical production to biomass production.
19. (original) The bio-fuel cell system according to claim 4 including reagent control means for controlling concentrations of the dissolved nutrients concentrations for varying microbial cultivation parameters in order to control a ratio of electrical production to biomass production.
20. (original) A bio-fuel cell system, comprising;
- a) a cathode compartment containing a cathode electrode, a pump for pumping a fluid containing oxygen and carbon dioxide into the cathode compartment;
  - b) an anode compartment containing an anode electrode with a fuel having a hydrogen constituent being pumped into said anode compartment, said anode compartment being separated from said cathode compartment by a membrane permeable to protons, a reaction at the anode electrode being electrochemical oxidation of the fuel to produce electrons ( $e^-$ ) and protons ( $\text{H}^+$ ), wherein protons ( $\text{H}^+$ ), formed by the oxidation of the fuel cross, wherein protons ( $\text{H}^+$ ), formed by the oxidation of the fuel cross the membrane into the cathode compartment; and
  - c) chemolithotrophic microorganisms immobilized on said cathode electrode, an aqueous solution containing substantially no iron, coating said chemolithotrophic microorganisms for maintaining a suitable humidity of the microbial cells, wherein a reaction at the cathode electrode is biological reduction of oxygen at the cathode electrode in a reaction given by  $\text{O}_2 + 4\text{H}^+ + 4e^- = 2\text{H}_2\text{O}$ , wherein electrons in that reaction are obtained by transfer from the cathode electrode to the attached microbial

cells, wherein electrical power is obtained by making electrical connection between a load and the anode and cathode electrodes.

21. (original) The bio-fuel cell system according to claim 20 wherein the chemolithotrophic microorganisms are immobilized on said cathode electrode containing a substantially chemically inert material, which facilitates microbial immobilization.
22. (original) The bio-fuel cell system according to claim 21 wherein the chemically inert material is one of silicon dioxide powder or gel, aluminum oxide (alumina) and calcium sulfate.
23. (currently amended) The bio-fuel cell system according to claim 20, ~~21 or 22~~ wherein the aqueous solution in contact with the chemolithotrophic microorganisms is a capillary layer coating the chemolithotrophic microorganisms and cathode, and wherein the fluid containing oxygen ( $O_2$ ) and carbon dioxide pumped into the cathode compartment is oxygen-containing gas such as air.
24. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22 or 23~~ wherein the fluid containing oxygen ( $O_2$ ) and carbon dioxide pumped into the cathode compartment is the aqueous solution containing oxygen ( $O_2$ ) and carbon dioxide dissolved therein.
25. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22, 23 or 24~~ wherein the membrane permeable to protons is a proton exchange membrane.
26. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22, 23 or 24~~ wherein the membrane permeable to protons is made of a substantially inert material having pores extending therethrough less than about 10 micrometers in diameter.

27. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22, 23, 24, 25 or 26~~ wherein the bioreactor and the cathode compartment contain dissolved nutrients for facilitating growth of the chemolithotrophic microorganisms.
28. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22, 23, 24, 25, 26 or 27~~ wherein the dissolved nutrients is one or more of ammonium sulfate, potassium phosphate, magnesium sulfate, potassium chloride, calcium nitrate, calcium chloride and sulfuric acid.
29. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22, 23, 24, 25, 26, 27 or 28~~ wherein the fuel having a hydrogen constituent is selected from the group consisting of hydrogen gas, methanol, methane and ethanol.
30. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22, 23, 24, 25, 26, 27 or 28~~ wherein the fuel having a hydrogen constituent is hydrogen gas ( $H_2$ ), and wherein the electrochemical oxidation reaction is oxidation of hydrogen at the anode electrode in a reaction given by  $2H_2 = 4H^+ + 4e^-$ , and so that an overall bio-fuel cell reaction is given by  $2H_2 + O_2 = 2H_2O$ .
31. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22, 23, 24, 25, 26, 27, 28, 29 or 30~~ wherein the chemolithotrophic microorganisms are *Acidithiobacillus ferrooxidans*.
32. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22, 23, 24, 25, 26, 27, 28, 29 or 30~~ wherein the chemolithotrophic microorganisms are selected from the group consisting of *Leptospirillum ferrooxidans*, *Acidimicrobium*, *Alicyclobacillus*, and *Sulfobacillus*.
33. (currently amended) The bio-fuel cell system according to claim 20, ~~21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32 or 33~~ wherein the cathode electrode is made from a chemically inert electrically conducting material.

34. (original) The bio-fuel cell system according to claim 33 wherein the cathode electrode includes a fibrous layer of a material selected from the group consisting of carbon, nickel and stainless steel.

35. (original) The bio-fuel cell system according to claim 33 wherein the cathode electrode includes a solid plate of a material selected from the group consisting of carbon, nickel and stainless steel.

36. (original) A bio-fuel cell system, comprising;

a) a fuel cell including a cathode compartment containing a cathode electrode, the cathode electrode including an Fe-copolymer containing a redox couple ( $\text{Fe}^{2+}/\text{Fe}^{3+}$ ), chemolithotrophic microorganisms being immobilized on the Fe-copolymer, with a reaction at the cathode electrode being reduction of ferric ions at the cathode electrode in a reaction given by  $4\text{Fe}^{3+} + 4e^- = 4\text{Fe}^{2+}$ , a pump for pumping a fluid containing oxygen ( $\text{O}_2$ ) and carbon dioxide ( $\text{CO}_2$ ) into the cathode compartment; and

b) an anode compartment containing an anode electrode with a fuel having a hydrogen constituent being pumped into said anode compartment, said anode compartment being separated from said cathode compartment by a membrane permeable to protons, a reaction at the anode electrode being electrochemical oxidation of the fuel to produce electrons ( $e^-$ ) and protons ( $\text{H}^+$ ), wherein protons ( $\text{H}^+$ ), formed by the oxidation of the fuel cross the membrane into the cathode compartment, and wherein the ferrous ions are oxidized by the chemolithotrophic microorganisms to ferric ions ( $\text{Fe}^{3+}$ ) in an aerobic oxidation reaction in the cathode compartment given by  $4\text{Fe}^{2+} + 4\text{H}^+ + \text{O}_2 = 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$ , wherein electrical power is obtained by making electrical connection between a load and the anode and cathode electrodes.

37. (original) The bio-fuel cell system according to claim 36 wherein the bioreactor and the cathode compartment contain dissolved nutrients for facilitating growth of the chemolithotrophic microorganisms.

38. (original) The bio-fuel cell system according to claim 37 wherein the dissolved nutrients is one or more of ammonium sulfate, potassium phosphate, magnesium sulfate, potassium chloride, calcium nitrate, calcium chloride and sulfuric acid.

39. (currently amended) The bio-fuel cell system according to claim 36, ~~37 or 38~~ wherein the membrane permeable to protons is a proton exchange membrane.

40. (currently amended) The bio-fuel cell system according to claim 36, ~~37 or 38~~ wherein the membrane permeable to protons is made of a substantially inert material having pores extending therethrough less than about 10 micrometers in diameter.

41. (currently amended) The bio-fuel cell system according to claim 36, ~~37, 38, 39 or 40~~ wherein the fluid containing oxygen is in a gaseous form.

42. (currently amended) The bio-fuel cell system according to claim 36, ~~37, 38, 39, 40 or 41~~ wherein the fluid containing oxygen ( $O_2$ ) and carbon dioxide ( $CO_2$ ) pumped into the cathode compartment is an aqueous solution containing oxygen ( $O_2$ ) and carbon dioxide ( $CO_2$ ) dissolved therein.

43. (currently amended) The bio-fuel cell system according to claim 36, ~~37, 38, 39, 40 or 41~~ wherein the fuel having a hydrogen constituent is selected from the group consisting of hydrogen gas, methanol, methane and ethanol.

44. (currently amended) The bio-fuel cell system according to claim 36, ~~37, 38, 39, 40 or 41~~ wherein the fuel having a hydrogen constituent is hydrogen gas, and wherein the electrochemical oxidation reaction is oxidation of hydrogen at the anode electrode in a reaction given by  $2H_2 = 4H^+ + 4e^-$ , and so that an overall bio-fuel cell reaction is given by  $2H_2 + O_2 = 2H_2O$ .

45. (currently amended) The bio-fuel cell system according to claim 36, ~~37, 38, 39, 40, 41, 42, 43 or 44~~ including voltage control means for applying and controlling a

voltage on the cathode electrode for controlling a ratio of electrical production to biomass production by varying microbial cultivation parameters.

46. (currently amended) The bio-fuel cell system according to claim 36, ~~37, 38, 39, 40, 41, 42, 43, 44 or 45~~ including reagent control means for controlling a ratio of  $\text{Fe}^{2+}/\text{Fe}^{3+}$  concentrations for varying microbial cultivation parameters in order to control a ratio of electrical production to biomass production.

47. (currently amended) The bio-fuel cell system according to claim 37 or ~~38~~ including reagent control means for controlling concentrations of the dissolved nutrients concentrations for varying microbial cultivation parameters in order to control a ratio of electrical production to biomass production.

48. (currently amended) The bio-fuel cell system according to claim 36, ~~37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47~~ wherein the chemolithotrophic microorganisms are *Acidithiobacillus ferrooxidans*.

49. (currently amended) The bio-fuel cell system according to claim 36, ~~37, 38, 39, 40, 41, 42, 43, 44, 45, 46 or 47~~ wherein the chemolithotrophic microorganisms are selected from the group consisting of *Leptospirillum ferrooxidans*, *Acidimicrobium*, *Alicyclobacillus*, and *Sulfobacillus*.

50. (currently amended) The bio-fuel cell system according to claim 36, ~~37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 or 49~~ wherein the cathode electrode is made from a chemically inert electrically conducting material.

51. (original) The bio-fuel cell system according to claim 50 wherein the cathode electrode includes a layer of a porous material selected from the group consisting of carbon, nickel and stainless steel.

52. (original) The bio-fuel cell system according to claim 50 wherein the cathode electrode includes a solid plate of a material selected from the group consisting of carbon, nickel and stainless steel.

53. (original) A bio-fuel cell system, comprising;

a) a cathode compartment containing a cathode electrode, chemolithotrophic microorganisms being immobilized on the cathode and in contact with an aqueous solution containing a salt of iron, with a reaction at the cathode electrode being reduction of ferric ions at the cathode electrode in a reaction given by  $4\text{Fe}^{3+} + 4\text{e}^- = 4\text{Fe}^{2+}$ , a pump for pumping a fluid containing oxygen ( $\text{O}_2$ ) and carbon dioxide into the cathode compartment; and

b) an anode compartment containing an anode electrode with a fuel having a hydrogen constituent being pumped into said anode compartment, said anode compartment being separated from said cathode compartment by a membrane permeable to protons, a reaction at the anode electrode being electrochemical oxidation of the fuel to produce electrons ( $\text{e}^-$ ) and protons ( $\text{H}^+$ ), wherein protons ( $\text{H}^+$ ) formed by the oxidation of the fuel cross the membrane into the cathode compartment, and wherein the ferrous ions ( $\text{Fe}^{2+}$ ) are oxidized by the chemolithotrophic microorganisms to ferric ions ( $\text{Fe}^{3+}$ ) in an aerobic oxidation reaction in the cathode compartment given by  $4\text{Fe}^{2+} + 4\text{H}^+ + \text{O}_2 = 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$ , wherein electrical power is obtained by making electrical connection between a load and the anode and cathode electrodes.

54. (original) The bio-fuel cell system according to claim 53 wherein the aqueous solution containing a salt of iron in contact with the chemolithotrophic microorganisms is a capillary layer coating the chemolithotrophic microorganisms and cathode, and wherein the fluid containing oxygen ( $\text{O}_2$ ) and carbon dioxide pumped into the cathode compartment is an oxygen-containing gas.

55. (original) The bio-fuel cell system according to claim 53 wherein the fluid containing oxygen ( $\text{O}_2$ ) pumped into the cathode compartment is the aqueous solution containing the salt of iron which has oxygen ( $\text{O}_2$ ) and carbon dioxide dissolved therein.

56. (currently amended) The bio-fuel cell system according to claim 53, ~~54 or 55~~ wherein the fuel having a hydrogen constituent is selected from the group consisting of hydrogen gas, methanol, methane and ethanol.

57. (original) The bio-fuel cell system according to claim 56 wherein the fuel having a hydrogen constituent is hydrogen gas, and wherein the electrochemical oxidation reaction is oxidation of hydrogen at the anode electrode in a reaction given by  $2H_2 = 4H^+ + 4e^-$ , and so that an overall bio-fuel cell reaction is given by  $2H_2 + O_2 = 2H_2O$ .

58. (currently amended) The bio-fuel cell system according to claim 53, ~~54, 55, 56 or 57~~ including voltage control means for applying and controlling a voltage on the cathode electrode for controlling a ratio of electrical production to biomass production by varying microbial cultivation parameters.

59. (currently amended) The bio-fuel cell system according to claim 53, ~~54, 55, 56, 57 or 58~~ wherein the chemolithotrophic microorganisms are *Acidithiobacillus ferrooxidans*.

60. (currently amended) The bio-fuel cell system according to claim 53, ~~54, 55, 56, 57 or 58~~ wherein the chemolithotrophic microorganisms are selected from the group consisting of *Leptospirillum ferrooxidans*, *Acidimicrobium*, *Alicyclobacillus*, and *Sulfobacillus*.

61. (currently amended) The bio-fuel cell system according to claim ~~52, 53, 54, 55, 56, 57 or 58~~ claim 53 wherein the cathode electrode is made from a chemically inert electrically conducting material.

62. (original) A method for generating electricity, comprising;  
a) pumping a fluid containing oxygen and carbon dioxide into a cathode compartment of a fuel cell, the cathode compartment including a cathode electrode and

a redox couple present therein with a reaction at the cathode electrode being reduction of a first member of the redox couple to a second member of the redox couple in a lower oxidation state;

b) pumping fuel into an anode compartment of the fuel cell containing an anode electrode with the fuel having a hydrogen constituent, said anode compartment being separated from said cathode compartment by a proton exchange membrane, a reaction at the anode electrode being electrochemical oxidation of the fuel to produce electrons ( $e^-$ ) and protons ( $H^+$ ), wherein protons ( $H^+$ ), formed by the oxidation of the fuel cross the proton exchange membrane into the cathode compartment; and

c) oxidizing the second member of the redox couple in the lower oxidation state back to the higher oxidation state by chemolithotrophic microorganisms in the presence of oxygen wherein electrical power in an electrical load is obtained by making electrical connection between the electrical load and the anode and cathode electrodes.

63. (original) The method according to claim 62 wherein the redox couple is  $Fe^{2+}/Fe^{3+}$ , and the wherein the reaction at the cathode electrode is reduction of ferric ions at the cathode electrode in a reaction given by  $4Fe^{3+} + 4e^- = 4Fe^{2+}$ , and wherein the chemolithotrophic microorganisms are contained in a bioreactor into which a fluid containing oxygen ( $O_2$ ) is pumped into the bioreactor, the bioreactor being in flow communication with the cathode compartment so that the aqueous solution containing ferrous ions ( $Fe^{2+}$ ) and protons ( $H^+$ ) is circulated from the cathode compartment to the bioreactor where the ferrous ions ( $Fe^{2+}$ ) are oxidized by the chemolithotrophic microorganisms to ferric ions ( $Fe^{3+}$ ) in an aerobic oxidation reaction given by  $4Fe^{2+} + 4H^+ + O_2 = 4Fe^{3+} + 2H_2O$ .

64. (original) The method according to claim 62 wherein the redox couple is  $Fe^{2+}/Fe^{3+}$ , and wherein the reaction at the cathode electrode is reduction of ferric ions at the cathode electrode in a reaction given by  $4Fe^{3+} + 4e^- = 4Fe^{2+}$ , and wherein the chemolithotrophic microorganisms are contained in the cathode compartment so that the ferrous ions ( $Fe^{2+}$ ) are oxidized by the chemolithotrophic microorganisms to ferric ions ( $Fe^{3+}$ ) in an aerobic oxidation reaction given by  $4Fe^{2+} + 4H^+ + O_2 = 4Fe^{3+} + 2H_2O$ .

65. (currently amended) The method according to claim 62, 63 or 64 wherein the chemolithotrophic microorganisms are *Acidithiobacillus ferrooxidans*.
66. (currently amended) The method according to claim 62, 63 or 64 wherein the chemolithotrophic microorganisms are selected from the group consisting of *Leptospirillum ferrooxidans*, *Acidimicrobium*, *Alicyclobacillus*, and *Sulfobacillus*.
67. (currently amended) The method according to claim 62, 63, 64, 65 or 66 wherein the fuel having a hydrogen constituent is selected from the group consisting of hydrogen gas, methanol, methane and ethanol.
68. (original) The bio-fuel cell system according to claim 67 wherein the fuel having a hydrogen constituent is hydrogen gas, and wherein the electrochemical oxidation reaction is oxidation of hydrogen at the anode electrode in a reaction given by  $2H_2 = 4H^+ + 4e^-$ , and so that an overall bio-fuel cell reaction is given by  $2H_2 + O_2 = 2H_2O$ .
69. (currently amended) The method according to claim 62, 63, 64, 65, 66, 67 or 68 including controlling a ratio of electrical production to biomass production by varying microbial cultivation parameters including an electrical potential of the cathode electrode, or by varying the ratio of  $Fe^{2+}/Fe^{3+}$  concentrations, or a combination of both.